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Lack of efflux mediated quinolone resistance in *Salmonella enterica* serovars Typhi and Paratyphi A

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Salmonella enterica serovars Typhi and Paratyphi A isolates from human patients in France displaying different levels of resistance to quinolones or fluoroquinolones were studied for resistance mechanisms to these antimicrobial agents. All resistant isolates carried either single or multiple target gene mutations (i.e., in *gyrA*, *gyrB*, or *parC*) correlating with the resistance levels observed. Active efflux, through upregulation of multipartite efflux systems, has also been previously reported as contributing mechanism for other serovars. Therefore, we investigated also the occurrence of non-target gene mutations in regulatory regions affecting efflux pump expression. However, no mutation was detected in these regions in both Typhi and Paratyphi isolates of this study. Besides, no overexpression of the major efflux systems was observed for these isolates. Nevertheless, a large deletion of 2334 bp was identified in the *acrS-acrE* region of all *S. Typhi* strains but which did not affect the resistance phenotype. As being specific to *S. Typhi*, this deletion could be used for specific molecular detection purposes. In conclusion, the different levels of quinolone or FQ resistance in both *S. Typhi* and *S. Paratyphi A* seem to rely only on target modifications.

Keywords: *Salmonella*, ciprofloxacin, transcriptional regulatory genes, *acrS*, efflux pumps

INTRODUCTION

Enteric fever caused by the human-adapted pathogens *Salmonella enterica* serovars Typhi (*S. Typhi*) and Paratyphi A (*S. Paratyphi A*), B, and C, remains a major health problem (Crump and Mintz, 2010). A global epidemiologic study estimated that during the year 2000 typhoid fever caused 21.7 million illnesses and 21,7000 deaths and paratyphoid fever caused 5.4 million illnesses (Crump et al., 2004). During the past decade *S. Paratyphi A* was responsible for a growing proportion of enteric fever in Asia (Ochiai et al., 2005; Crump and Mintz, 2010). Enteric fever being associated with poor sanitation and unsafe food and water, it particularly affects children and adolescents in developing countries of Asia, Africa and Latin America (Crump et al., 2004; Bhan et al., 2005; Crump and Mintz, 2010). In developed countries, patients are most often ill-returned travellers or migrant workers (Bhan et al., 2005; Connor and Schwartz, 2005; Hassing et al., 2013).

To treat these infections, fluoroquinolones (FQ) and third-generation cephalosporins have been considered as first-line drugs, owing to the resistance to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole that appeared during the 1980s (Hassing et al., 2011, 2013). Multidrug resistance (MDR) in *S. Typhi* is encoded mainly by resistance genes carried by large conjugative plasmids and has been reported worldwide (Le et al., 2007). As a consequence of a widespread FQ usage, *S. Typhi* and *S. Paratyphi A* isolates resistant to nalidixic acid (NAL^R, minimum inhibitory concentration [MIC] > 16 mg/L) and with decreased susceptibility to ciprofloxacin (CIP^{DS}, MIC 0.125–1.0 mg/L) have also emerged. Such NAL^R-CIP^{DS} *S. Typhi*

and *S. Paratyphi A* have been isolated in endemic areas and also in developed countries (Roumagnac et al., 2006; Le et al., 2007; Gaborieau et al., 2010; Accou-Demartin et al., 2011; Hassing et al., 2011, 2013).

Resistance to quinolones in *Salmonella* spp. is mostly attributed to point mutations in the quinolone resistance-determining regions (QRDRs) of the target genes *gyrA*, *gyrB*, *parC*, and *parE* (Cloeckaert and Chaslus-Dancla, 2001; Piddock, 2002; Velge et al., 2005; Giraud et al., 2006). For the *gyrA* gene, coding the A subunit of DNA gyrase, a single mutation resulting in an amino acid substitution at the position 83 (Serine to Phenylalanine or to Tyrosine) or at the position 87 (Aspartic acid to Asparagine or Glycine) has been the most frequently described in NAL^R-CIP^{DS} *S. Typhi* and *S. Paratyphi A* isolates (Bhan et al., 2005; Renuka et al., 2005; Le et al., 2007; Gaborieau et al., 2010; Hassing et al., 2011). A second mutation leading to the amino acid change at the position 80 (Serine to Isoleucine or to Arginine) of the ParC subunit of topoisomerase IV was described to increase the CIP MIC (≥ 0.5 mg/L) in *S. Typhi* and *S. Paratyphi A* human isolates from India (Gaïnd et al., 2006). Whereas three mutations, i.e., a double mutation in *gyrA* at both codons 83 and 87 and one mutation in *parC*, were shown to confer CIP resistance (MIC > 1 mg/L) in *S. Typhi* and *S. Paratyphi A* human isolates from India or from Taiwan (Gaïnd et al., 2006; Lee et al., 2013).

Moreover, the varying levels of CIP resistance observed in *S. Typhi* and *S. Paratyphi A* isolates with only a single *gyrA* mutation suggest that other mechanisms could be involved in quinolone resistance in this serovar (Renuka et al., 2005).

Resistance to FQ in *S. Typhimurium* has also been attributed to active efflux mechanism, due to overproduction of the AcrAB-TolC efflux system (Giraud et al., 2000, 2006; Cloeckaert and Chaslus-Dancla, 2001; Piddock, 2006). We have previously reported the contribution of the AcrAB-TolC efflux system in resistance to FQ in several MDR epidemic clones of *S. Typhimurium*, such as *S. Typhimurium* of phage types DT204 or DT104 (Baucheron et al., 2002, 2004a,b). Among the chromosomal loci affecting AcrAB-TolC expression, the *ramRA* locus appears to be the most important in *Salmonella* spp. (Abouzeed et al., 2008; Kehrenberg et al., 2009). *ramR* encodes a repressor protein (RamR) belonging to the TetR family of repressor proteins, and has been shown to be the local repressor protein of *ramA* transcription (Abouzeed et al., 2008; Baucheron et al., 2012); while *ramA* encodes a transcriptional activator protein (RamA) belonging to the AraC/XylS family of regulatory proteins (Nikaido et al., 2008; Bailey et al., 2010). The latter is involved in upregulating expression of the AcrAB-TolC system (Nikaido et al., 2008; Bailey et al., 2010). Several mutations in *ramR* or its binding site upstream of *ramA*, affecting expression of this efflux system, have been detected in clinical isolates of serovar Typhimurium or Kentucky and of minor serovars Hadar, Infantis, Livingstone, or Schwarzengrund (Abouzeed et al., 2008; Kehrenberg et al., 2009;

Hentschke et al., 2010; Akiyama and Khan, 2012; Baucheron et al., 2013).

In the present study, we have characterized mechanisms involved in resistance to quinolones or fluoroquinolones in 21 *S. Typhi* and *S. Paratyphi A* strains displaying different levels of resistance to these drugs and isolated from patients in France during the period 1997–2008. For a subset of strains, with suspected increased efflux activity, we investigated the occurrence of mutations in the global *ram*, *sox* and *mar* regulatory loci of AcrAB-TolC, and in the local *acrR* and *acrS* repressor genes of the AcrAB and AcrEF pumps, respectively (Abouzeed et al., 2008; Kehrenberg et al., 2009).

MATERIALS AND METHODS

BACTERIAL STRAINS

The twenty one strains including 16 *S. Typhi* and 5 *S. Paratyphi A* selected for this study were collected by the French National Reference Center for *Salmonella*, Institut Pasteur, Paris, France. They were isolated in France from travellers or migrants between 1997 and 2008 (Table 1). The selection was made to obtain diversity in terms of geographic origin, year of isolation, genetic lineages (haplotype for *S. Typhi*; Roumagnac et al., 2006), and phenotype of resistance to quinolones (Table 1).

Table 1 | *Salmonella enterica* serovars Typhi and Paratyphi A strains analyzed in this study.

| Strain | Country | Year of isolation | Haplo type | Antimicrobial resistance pattern | MIC (mg/L) | | Substitution(s) in the QRDR of: | | | | AcrA production ratio* | |
|------------------------|---------------|-------------------|------------|----------------------------------|------------|-------|---------------------------------|-------|------|-------|------------------------|--|
| | | | | | NAL | CIP | GyrA | GyrB | ParC | ParE | | |
| SALMONELLA TYPHI | | | | | | | | | | | | |
| 06-423 | India | 2006 | ND | Pansusceptible | 4 | 0.015 | WT | WT | WT | WT | 1 | |
| 06-426 | India | 2006 | ND | CIP ^{DS} | 16 | 0.125 | WT | S464Y | WT | WT | 1 | |
| 02-1180 | India | 2002 | H45 | NALCIP ^{DS} | 64 | 0.125 | D87G | WT | WT | WT | 1 | |
| 05-3275 | Morocco | 2005 | H6 | NALCIP ^{DS} | 64 | 0.125 | D87N | WT | WT | WT | 1 | |
| 4(02)MB | Vietnam | 1997 | H58 | ASCSulTmptTeNAL | 128 | 0.03 | S83Y | WT | WT | WT | 0.5 | |
| 222(97)MN | Vietnam | 1996 | ND | ASCSulTmptTeNALCIP ^{DS} | 128 | 0.125 | S83F | WT | WT | WT | 0.5 | |
| 43(97)MN | Vietnam | 1996 | H63 | ASCSulTmptTeNALCIP ^{DS} | 128 | 0.125 | S83F | WT | WT | WT | 0.5 | |
| 98-3139 | Mexico | 1998 | H50 | NALCIP ^{DS} | 128 | 0.125 | S83F | WT | WT | WT | 0.5 | |
| 02-7744 | India | 2002 | H52 | NALCIP ^{DS} | 128 | 0.125 | S83F | WT | WT | WT | 0.5 | |
| 226(97)MN | Vietnam | 1996 | H61 | ASCSulTmptTeNALCIP ^{DS} | 128 | 0.25 | S83F | WT | WT | WT | 0.5 | |
| 97-2307 | India | 1997 | H63 | NALCIP ^{DS} | 256 | 0.125 | S83F | WT | WT | WT | 0.5 | |
| 318(98)MB | Vietnam | 1998 | H58 | ASCSulTmptTeNALCIP ^{DS} | 512 | 0.25 | S83Y | WT | WT | WT | 1 | |
| 39(98)MN | Vietnam | 1998 | H58 | ASCSulTmptTeNALCIP ^{DS} | 512 | 0.25 | S83F | WT | WT | WT | 1 | |
| 4(02)MN | Vietnam | 2000 | H58 | ASCSulTmptTeNALCIP ^{DS} | 1024 | 0.25 | S83F | WT | WT | D420N | 1 | |
| 5(04)MN | Vietnam | 2004 | ND | NALCIP ^{DS} | 1024 | 0.25 | S83F | WT | WT | D420N | 1 | |
| 04-2176 | India | 2004 | H58 | SSpSulTmptTeNALCIP | 1024 | 8 | S83F; D87N | WT | S80I | WT | 1 | |
| SALMONELLA PARATYPHI A | | | | | | | | | | | | |
| 08-8903 | Senegal | 2008 | | Pansusceptible | 8 | 0.030 | WT | WT | WT | WT | 2 | |
| 07-6329 | Burkina Faso | 2007 | | CIP ^{DS} | 16 | 0.25 | WT | S464F | WT | WT | 2 | |
| 05-208 | India | 2005 | | NALCIP ^{DS} | 256 | 0.50 | S83F | WT | WT | WT | 2 | |
| 08-4271 | Guinea Bissau | 2008 | | NACIP ^{DS} | 1024 | 1 | S83F | WT | WT | WT | 2 | |
| 08-2580 | India | 2008 | | NALCIP ^{DS} | 1024 | 1 | S83F | WT | WT | WT | 3 | |

*AcrA expression was measured by dot blotting with an anti-AcrA polyclonal antibody.

ND, not determined.

WT, wild type.

A, amoxicillin; S, streptomycin; Sp, spectinomycin; C, chloramphenicol; Sul, sulfamethoxazole; Tmp, trimethoprim; Te, tetracycline; NAL, nalidixic acid; CIP, ciprofloxacin; CIP^{DS}, decreased susceptibility to ciprofloxacin.

D, aspartic acid; F, phenylalanine; G, glycine; I, isoleucine; N, asparagine; S, serine; Y, tyrosine.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility was investigated by the standard disk diffusion method according to the recommendations of the Antibigram Committee of the French Society for Microbiology (CA-SFM) (www.sfm-microbiologie.org/). The MICs of NAL and CIP were determined by the standard agar doubling dilution method as described previously (Baucheron et al., 2002). The NAL^R isolates were defined as having a MIC > 16 mg/L. The CIP^R isolates were defined as having a MIC > 1 mg/L and CIP^{DS} isolates as having a MIC comprised between 0.125 and 1.0 mg/L (Accou-Demartin et al., 2011; Hassing et al., 2013). MICs of these antibiotics were also determined in the presence of the efflux pump inhibitor Phe-Arg- β -naphthylamide (PA β N, Sigma) at the following concentrations: 10, 20, 30, 40, 50, and 60 mg/L.

ASSESSMENT OF TARGET-AFFECTING MECHANISMS

Mutations in the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* genes were detected as described previously (Le et al., 2007; Song et al., 2010; Accou-Demartin et al., 2011).

The search of plasmid-mediated quinolone resistances genes, *qnrA*, *qnrB*, *qnrS*, *qnrD*, *aac(6')*-Ib-cr, and *qepA* was performed as described previously (Accou-Demartin et al., 2011).

ASSESSMENT OF EFFLUX MECHANISMS

Efflux pump production was assessed by dot blot using an anti-AcrA polyclonal antibody as described previously (Abouzeed et al., 2008). Occurrence of mutations affecting *acrAB*, *acrEF*, and *tolC* expression was determined by PCR and sequencing the regulatory regions *ramR-ramA*, *acrR-acrA*, *marC-marO-marR-marA*, *soxS-soxR*, and *acrS-acrE* using primers listed in (Table 2).

Table 2 | Primers used for PCRs.

| Primer used and target region | Primer | Nucleotide position relative to the LT2 strain genome sequence * | Oligonucleotide sequence(s) (5'–3') | Size (bp) | Annealing temp (°C) | Reference |
|-------------------------------|--------|--|-------------------------------------|-----------|---------------------|------------------------|
| DETECTION OF MUTATIONS | | | | | | |
| ramR-ramA | ram5 | 638085 | TCGGTAAAAGGCAGTTCAG | 958 | 60 | Baucheron et al., 2013 |
| | ramA6 | 639042 | GTCGATAACCTGAGCGGAAA | | | |
| acrR-acrA | acrR1 | 533463 | CAGTGGTTCGGTTTTAGTG | 992 | 58 | Olliver et al., 2005 |
| | acrR2 | 534454 | ACAGAATAGCGACACAGAAA | | | |
| marC-marO-marR-marA | marR1 | 1597459 | CAGTGTTGCGTCTGGACATC | 787 | 60 | Baucheron et al., 2013 |
| | marR2 | 1598245 | GCTAACGGGAGCAGTACGAC | | | |
| soxS-soxR | sox1 | 4503970 | CTACAGGCGGTGACGGTAAT | 915 | 60 | Baucheron et al., 2013 |
| | sox2 | 4504884 | CGGCGCTTTAGTTTTAGGTG | | | |
| acrS-acrE | acrS3 | 3559106 | AAAACGAACGGGAACATGATG | 2874 *** | 58 | This study |
| | acrS4 | 3561978 | ACAAACATACCGGGAAGCAG | | | |
| qRT-PCR | | | | | | |
| gmk | gmk-f | 3933294 | TTGGCAGGGAGGCGTTT | 62 | 60 | Baucheron et al., 2012 |
| | gmk-r | 3933355 | GCGCGAAGTGCCGTAGTAAT | | | |
| gyrB | gyrB-f | 4040275 | TCTCCTCACAGACCAAAGATAAGCT | 81 | 60 | Baucheron et al., 2012 |
| | gyrB-r | 4040195 | CGCTCAGCAGTTCCGTTTCATC | | | |
| rrs | rrs-f | NA** | CCAGCAGCCGCGGTAAT | 57 | 60 | Baucheron et al., 2012 |
| | rrs-r | NA** | TTTACGCCCAGTAATCCGATT | | | |
| ramA | ramA-f | 639180 | GCGTGAACGGAAGCTAAAAC | 167 | 60 | Baucheron et al., 2012 |
| | ramA-r | 639346 | GGCCATGCTTTTCTTTACGA | | | |
| ramR | ramR-f | 638623 | TAACGCAGGTGTTGCAGAAG | 192 | 64 | Baucheron et al., 2012 |
| | ramR-r | 638432 | TGGTTCAGACCCCAACTGAT | | | |
| acrA | acrA-f | 533120 | GAAACCGCACGTATCAACCT | 220 | 60 | Baucheron et al., 2012 |
| | acrA-r | 532901 | CCTGTTTCAGCGAACCATT | | | |
| acrB | acrB-f | 531348 | TCGTGTTCTGGTGATGTACCT | 68 | 66 | Baucheron et al., 2012 |
| | acrB-r | 531281 | AACCGCAATAGTCGGAATCAA | | | |
| acrF | acrF-f | 3563042 | GCTCTGTCTGCCATCTCAAAGA | 70 | 66 | This study |
| | acrF-r | 3563111 | CGCGCTACAACGTTATAGTTTTCA | | | |
| tolC | tolC-f | 3349107 | GCCCGTGCGCAATATGAT | 67 | 60 | Baucheron et al., 2012 |
| | tolC-r | 3349173 | CCGCGTTATCCAGGTTGTTG | | | |

*GenBank NC_0031971.

**NA: Not Applicable due to the number of copies of this gene in *Salmonella*.

***2874 bp for *S. Typhimurium* or *S. Paratyphi A* and 539 bp for *S. Typhi* (see Figure 1).

Transcription levels of efflux related genes *acrA*, *acrB*, *acrF*, *tolC*, *ramA*, and *ramR* were done by quantitative reverse transcription-PCR (qRT-PCR) as described previously (Baucheron et al., 2012; Giraud et al., 2013). Primers used for qRT-PCR are listed in (Table 2).

RESULTS AND DISCUSSION

RESISTANCE PHENOTYPES AND TARGET-AFFECTING MECHANISMS

The twenty one *S. Typhi* and *S. Paratyphi A* strains of this study were isolated in France but acquired abroad, mainly in Asia and Africa (Table 1). Among the *S. Typhi* strains, all but two were NAL^R (MIC > 16 mg/L). One of the two strains was pan-susceptible and the second was CIP^{DS} but only categorized as intermediate for NAL (NAL^I, MIC 16 mg/L). Of the 14 NAL^R strains, one was CIP^R, 12 were CIP^{DS} and one was CIP^S (MIC 0.03 mg/L). Eight NAL^R *S. Typhi* strains were also multidrug resistant. The majority of the NAL^R *S. Typhi* strains belonged to haplotype H58 which had emerged in Southern Asia during the mid 1990s (Roumagnac et al., 2006; Le et al., 2007). Among the *S. Paratyphi A* strains, all but two were NAL^R. One of the two strains was pansusceptible and the second was NAL^I-CIP^{DS}. The three others were NAL^R-CIP^{DS}.

As shown in Table 1, both NAL^I-CIP^{DS} had a mutation resulting in an amino acid substitution at position 464 of GyrB: serine to tyrosine for the *S. Typhi* isolate and serine to phenylalanine for the *S. Paratyphi A* isolate. The most frequent mechanism of resistance of NAL^R-CIP^{DS} ($n = 17$) and NAL^R-CIP^S ($n = 1$) strains, whatever the serovar, was a substitution at position 83 (serine to phenylalanine, $n = 12$, 66.6%) of GyrA. Other GyrA modifications were observed at position 83 (serine to tyrosine) in two isolates or at position 87 (aspartic acid to glycine, aspartic acid to asparagine) in one isolate for each. As described previously, a single substitution in GyrA was associated with resistance to

nalidixic acid and decreased susceptibility to CIP (Bhan et al., 2005; Le et al., 2007; Gaborieau et al., 2010; Hassing et al., 2011). One exception was the *S. Typhi* strain 4 (02)MB, which was NAL^R-CIP^S (and not CIP^{DS}) despite a mutation in *gyrA* resulting in substitution serine to tyrosine at position 83.

Additional substitutions were found in ParE of 2 NAL^R-CIP^{DS} *S. Typhi* strains that led to amino acid substitution aspartic acid to asparagine at position 420. In both cases, a 2-fold increase of NAL MICs was observed.

In the CIP^R *S. Typhi* isolate, three mutations leading to a double substitution in GyrA at positions 83 (serine to phenylalanine) and 87 (aspartic acid to asparagine) and one substitution at the position 80 of ParC (serine to isoleucine), as observed in previous studies (Renuka et al., 2005; Gaind et al., 2006; Lee et al., 2013).

The NAL^R and CIP^{DS} *S. Typhi* and *S. Paratyphi A* strains harboring a single substitution in GyrA showed various values for NAL (64–1024 mg/L) and CIP (0.03–0.5 mg/L) MICs which suggested the presence of other mechanisms of resistance. Since the plasmid-mediated quinolone resistance-conferring genes *qnrA*, *qnrB*, *qnrD* or *qnrS*, *qepA*, and *aac(6′)-Ib-cr* were not detected, we investigated the role of the AcrAB-TolC efflux system.

INVOLVEMENT OF EFFLUX

None of the *S. Typhi* strains showed significant AcrA overproduction by dot blot, but nevertheless all *S. Paratyphi A* isolates showed a 2 or 3-fold increased AcrA production relative to the susceptible *S. Typhi* isolate (Table 1). Thus, overproduction of AcrA seems not to be involved in CIP^{DS} isolates compared with the susceptible isolates of *S. Typhi* or *S. Paratyphi A*. In presence of the efflux pump inhibitor PAβN (20 or 40 mg/L), the CIP MICs similarly decreased (4 or 8-fold) in CIP^{DS} and in susceptible strains (Table 3 and data not shown), which is in accordance with previous studies on *S. Typhimurium* and corresponds to

Table 3 | Study of efflux in a subset of *Salmonella enterica* serovars Typhi and Paratyphi A strains.

| Strain | Antimicrobial resistance pattern ^a | MIC (mg/L) ^b | | Substitution(s) in the QRDR of ^c : | | | | <i>acrSE</i> sequencing | Transcription level of: | | | | | |
|------------------------|---|-------------------------|---------------|---|-------|------|------|-------------------------|-------------------------|-------------|-------------|-------------|-------------|-------------|
| | | NAL | CIP | GyrA | GyrB | ParC | ParE | | <i>acrA</i> | <i>acrF</i> | <i>acrB</i> | <i>tolC</i> | <i>ramA</i> | <i>ramR</i> |
| SALMONELLA TYPHI | | | | | | | | | | | | | | |
| 06–423 | Pansusceptible | 4 | 0.015 [0.004] | WT | WT | WT | WT | Deletion ^d | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 02–1180 | NALCIP ^{DS} | 64 | 0.125 [0.015] | D87G | WT | WT | WT | Deletion ^d | 1.5 | 0.7 | 0.5 | 0.8 | 0.9 | 1.4 |
| 05–3275 | NALCIP ^{DS} | 64 | 0.125 [0.030] | D87N | WT | WT | WT | Deletion ^d | 0.5 | 1.4 | 0.2 | 0.5 | 0.3 | 0.7 |
| 97–2307 | NALCIP ^{DS} | 256 | 0.125 [0.030] | S83F | WT | WT | WT | Deletion ^d | 1.7 | 0.8 | 0.8 | 0.7 | 1.9 | 2.2 |
| 04–2176 | SSpSulTmptTeNALCIP | 1024 | 8 [2] | S83F; A87N | WT | S80I | WT | Deletion ^d | 1.5 | 0.8 | 1.3 | 0.9 | 1.2 | 2.2 |
| SALMONELLA PARATYPHI A | | | | | | | | | | | | | | |
| 08–8903 | Pansusceptible | 8 | 0.030 [0.008] | WT | WT | WT | WT | WT | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 07–6329 | CIP ^{DS} | 16 | 0.25 [0.060] | WT | S464F | WT | WT | WT | 1.6 | 1.4 | 1.0 | 1.0 | 1.0 | 1.2 |
| 05–208 | NALCIP ^{DS} | 256 | 0.50 [0.030] | S83F | WT | WT | WT | WT | 1.3 | 0.9 | 1.4 | 1.0 | 1.1 | 1.0 |
| 08–4271 | NALCIP ^{DS} | 1024 | 1 [0.25] | S83F | WT | WT | WT | WT | 1.2 | 1.1 | 0.7 | 1.4 | 1.3 | 1.3 |
| 08–2580 | NALCIP ^{DS} | 1024 | 1 [0.25] | S83F | WT | WT | WT | WT | 2.0 | 1.6 | 1.4 | 1.4 | 2.0 | 1.3 |

^a S, streptomycin; Sp, spectinomycin; Sul, sulfamethoxazole; Tmp, trimethoprim; Te, tetracycline; NAL, nalidixic acid; CIP, ciprofloxacin; CIP^{DS}, decreased susceptibility to ciprofloxacin.

^b Values in brackets are MICs in the presence of the efflux pump inhibitor PAβN at 40 mg/L.

^c WT, wild type; D, aspartic acid; F, phenylalanine; G, glycine; I, isoleucine; N, asparagine; S, serine.

^d 2334 bp deleted.

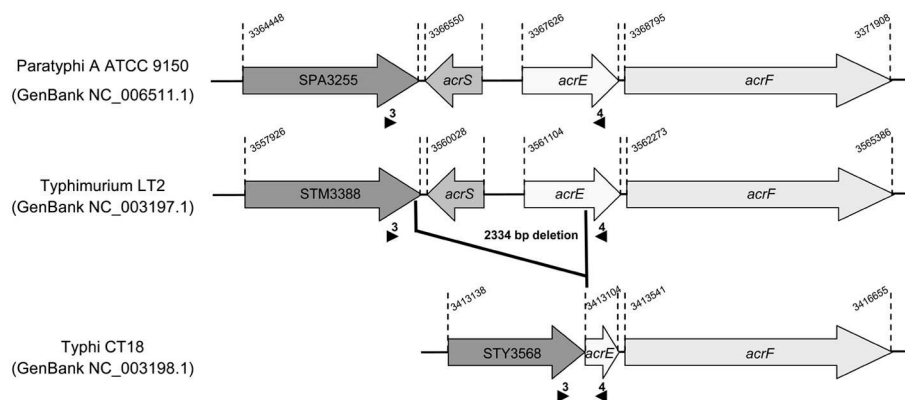


FIGURE 1 | Deletion identified in the *acrSE* region relative to the genome sequence of *S. Typhi* strain CT18 (GenBank NC_003198.1).

The sequence of the *acrSE* region of *S. Typhi* strain CT18 is compared to those of *S. Paratyphi* A strain ATCC9150 (GenBank NC_006511.1) and

S. Typhi strain LT2 (GenBank NC_003197.1). Small black arrows indicate primers positions used for PCR to amplify and sequence the *acrSE* region. The 2334 bp chromosomal deletion was found in all *S. Typhi* strains studied.

a decrease of resistance level observed for *acrB* or *tolC* deletion mutants (Baucheron et al., 2002, 2004b).

Despite a lack of evidence of increased efflux in the resistance phenotype, we measured by qRT-PCR the transcription levels of efflux related genes *acrA*, *acrF*, *acrB*, *tolC*, *ramA*, and *ramR* in CIP^{DS} non-MDR strains and in the CIP^R strain. No differences were detected in the transcription levels of these genes, between susceptible, CIP^{DS} and CIP^R strains, whatever the serovar (Table 3). In addition, no mutations were detected in the regulatory regions of the AcrAB-TolC efflux system. However, during the screening of the regulatory regions, we identified a single large deletion of 2334 bp in the *acrS*-*acrE* region of all *S. Typhi* strains, including the susceptible one (Table 3). This deletion encompassed the *acrS* gene, that encodes a transcriptional repressor, and a large part of the *acrE* gene that encodes the AcrE periplasmic lipoprotein, which is homologous to AcrA (Olliver et al., 2005). This 2334 bp deletion was also observed in the *acrS*-*acrE* region of sequenced genomes of MDR *S. Typhi* CT18 strain (Parkhill et al., 2001) and pansusceptible TY2 strain (Deng et al., 2003) (Figure 1). Previously, it has been shown that *acrS* deletion in *S. Typhimurium* does not affect *acrEF* expression (Olliver et al., 2005). Similarly the natural *acrSE* deletion detected in *S. Typhi* had no impact on the *acrF* transcription level as observed in this study. To our knowledge, this is the first description of such a natural *acrS*-*acrE* chromosomal deletion and seems specific to *S. Typhi* since it was not detected in all currently sequenced genomes of the other serovars (not shown).

CONCLUSIONS

The main mechanisms involved in quinolone or FQ resistance in both *S. Typhi* and *S. Paratyphi* A are target modifications. In contrast to what is seen in enteric pathogenic serovars, such as Typhimurium or the emerging CIP^R Kentucky ST198 clone (Baucheron et al., 2013), increased efflux pump production-mediated mechanisms seem to be totally absent in both *S. Typhi* and *S. Paratyphi* A. The deletion identified in the *acrSEF* region, although not involved in the resistance phenotype, may be helpful for the specific detection of *S. Typhi*.

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